ILLINOIS ENVIRONMENTAL PROTECTION AGENCY

1021 NORTH GRAND AVENUE EAST, P.O. BOX 19276, SPRINGFIELD, ILLINOIS 62794-9276 • (217) 782-2829

BRUCE RAUNER, GOVERNOR LISA BONNETT, DIRECTOR

217/558/2012

January 28, 2016

Mike Elias
Health and Environmental Safety
Office of Water (Mail Code 4304T)
United State Environmental Protection Agency
1200 Pennsylvania Ave. NW
Washington DC 20460

RE: Comments on the National Water Quality Criteria for Cadmium
Docket ID No. EPA-HQ-OW-2015-0735

Dear Mr. Elias:

Under this cover Illinois Environmental Protection Agency submits comments on the draft National Criteria for Cadmium. Specifically, we find that the proposed chronic criterion is based on a flawed toxicity test on the amphipod *Hyalella azteca*.

The following document explains our position that the *Hyalella* test used in the criterion derivation should be repeated using current feeding procedures that are proven to cause better growth and reproduction. As *Hyalella* was the most sensitive species in the criterion derivation, it is of utmost importance that this test portrays an accurate picture of the toxicity of cadmium. If time does not permit the repeat of this test, the chronic criterion should be recalculated with the *Hyalella* data removed.

Should you have any questions regarding our comments, please direct them to Brian Koch of my staff at the letterhead phone number. Thank you for considering our comments. We look forward to discussing the chronic cadmium criterion and *Hyalella* testing with you in the future.

Sincerely,

Marcia T. Willhite
Chief
Bureau of Water

cc: Candice Bauer, USEPA Region 5

Comments of the Illinois Environmental Protection Agency Regarding the Draft National Water Quality Criteria for Cadmium Docket ID No. EPA-HQ-OW-2015-0753 January 28, 2016

The Illinois Environmental Protection Agency ("IEPA") has reviewed the draft 2015 cadmium criteria document and supporting materials and has significant concerns regarding the development of the freshwater chronic criterion. Specifically, the IEPA questions the validity of results obtained from the 2000 USGS study (Ingersoll and Kemble 2001) that assessed the chronic toxicity of cadmium to the reproduction of *Hyalella azteca*, a freshwater amphipod. While the IEPA acknowledges and commends the improvements USEPA has made in the assessment and analysis of *Hyalella* sp. data compared to the 2001 cadmium criteria document, specifically in regards to the sensitivity of this organism to the presence/absence of chloride and bromide in culture and test water, the IEPA contends that the feeding regime employed in the 2000 USGS study was deficient by today's standards and likely resulted in malnourished, stressed test organisms. Given that *Hyalella azteca* is identified as the most chronically sensitive test organism within the 2015 cadmium criteria document (due to the reproductive endpoint ascertained from the 2000 USGS study), the resulting hardness-based chronic cadmium criterion is primarily driven by, and hinges on, this endpoint. Thus, it is crucial that the true chronic sensitivity of *Hyalella azteca* to cadmium be confidently ascertained before adoption of the criterion.

Since becoming aware of the 2015 draft cadmium criteria, the IEPA has participated in several discussions with Region 5 USEPA, USEPA Office of Science and Technology, and state protection agency personnel regarding the questioned validity of the chronic endpoint developed for *Hyalella azteca.* The concerns raised by the IEPA have yet to be resolved and, at this juncture, appear to only be resolvable through a retest of 2000 USGS study. The IEPA has expressed to USEPA its support of a retest by the same laboratory and same study authors, with one exception being an improved feeding regime and potentially a modified cadmium dilution series. While USEPA Office of Science and Technology and the study authors have stated that they stand behind the test results, it was reported to the IEPA by USEPA Region 5 staff that USEPA Office of Science and Technology has looked into, and seemingly would support, the commissioning of a retest of the 2000 USGS study. However, the IEPA has subsequently been notified that a retest is infeasible due to time constraints, as a court ordered deadline is in place that mandates the criteria to be adopted by March 30, 2016. Given that chronic *Hyalella azteca* tests are conducted over a duration of 42 days, the IEPA concurs that a retest prior to the March 30, 2016 deadline is infeasible. At this time, the IEPA is in support of the adoption of the acute cadmium criterion as proposed, but requests a one year extension be afforded for the adoption of the chronic criterion. A one year extension would allow for a retest on *Hyalella azteca* using present day feeding recommendations and would allow for revisions to be made to the chronic criterion and the documentation associated with it. If the extension cannot be granted, removal of *Hyalella azteca* from the chronic dataset and a recalculation of the chronic criterion is the only acceptable alternative.

Expounding upon the abbreviated comments provided above, the IEPA provides the following detailed comments and questions regarding the 2000 USGS study on *Hyalella azteca*:

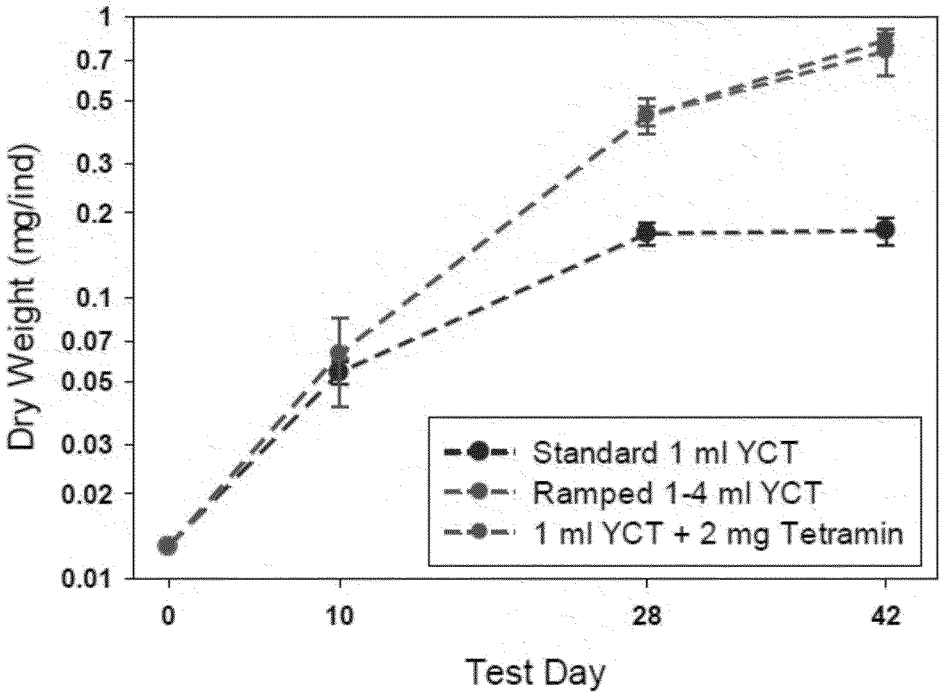
Test organisms in the 2000 USGS study were underfed and/or fed improper diets compared to current methods

The IEPA has long known of the difficulties associated with the culturing and testing of *Hyalella azteca*, which were learned through years of toxicity testing on this organism for use in water quality standards development. While the IEPA does not have its own laboratory or personnel for conducting aquatic toxicity testing, the IEPA has generated acute and chronic toxicity data through a longstanding contractual partnership with Dr. David Soucek, an aquatic toxicologist at the Illinois Natural History Survey who is considered a renowned researcher in the field of *Hyalella azteca* culturing and testing. Dr. Soucek has been part of a working group, which also includes researchers at USGS, USEPA, and

Environment Canada, that is tasked with updating standardized test methods for this organism. While substantial efforts initially focused on the appropriate culture/test water, including the importance of bromide and chloride as essential micronutrients in culture/test water, more recent phases of research have centered on the appropriate diet for these organisms. Dr. Soucek was interested in determining the appropriate amounts of food for test organisms without resulting in excess amounts that lead to dissolved oxygen problems, which in turn has led to much improved growth of test organisms compared to earlier diet regimes. The diet used in the 2000 USGS study consisted of a ration of 1.0 ml YCT/d, whereas Dr. Soucek's recent research finds that a diet consisting of Tetramin supplemented with diatoms results in greatly improved growth and reproduction of *Hyalella azteca* compared to YCT-only diets (Soucek et al. 2016, in press). For example, whereas the controls in the 2000 USGS cadmium test only reached an average weight of 0.27 mg/individual, Dr. Soucek has achieved growth rates exceeding 1.0 mg/individual using a Tetramin-based diet (Soucek et al. 2016, in press). Please see Table 3 from Soucek et al. 2016 (in press), provided as Attachment 1 within this comment letter, for further documentation of the disparity between the growth rates observed with YCT-based diets and enhanced, present day diets.

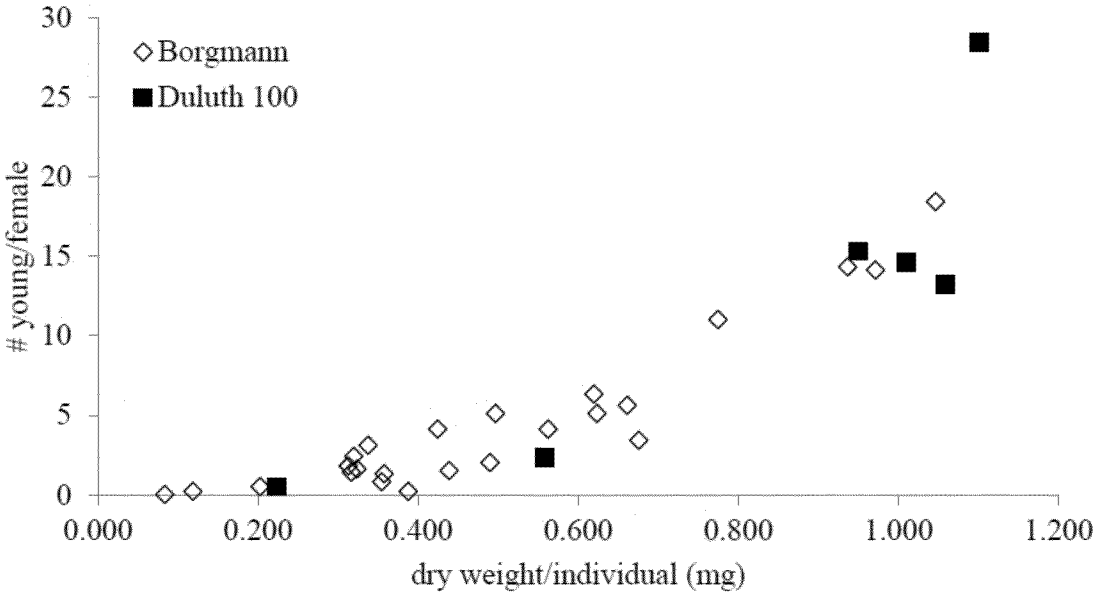
By today's standards for *Hyalella azteca* culturing and testing, the test organisms in the 2000 USGS were fed a suboptimal diet that not only restricted growth, but undoubtedly restricted the reproductive success of test organisms. In Appendix K of the draft 2015 cadmium criteria document, EPA concedes that growth of organisms fed 1 ml YCT/d would have reduced growth compared to organisms fed increased amounts of YCT or 1 ml YCT/d supplemented with Tetramin, yet states "*However, this limited growth does not seem to be so stressful as to reduce long-term survival, and reproduction still occurs though at lower rates than higher rations.*" Appendix K also includes a figure (Figure 6, inserted below) that depicts the discrepancies in growth achieved by *Hyalella azteca* when fed using the 2000 USGS diet (1 ml YCT/d) vs increased amounts of YCT or 1 ml YCT supplemented with Tetramin.

Figure 6. Growth rates of *Hyalella* reared on standard (EPA or ASTM 2000) ration of 1 ml YCT/d or on alternate rations (D.R. Mount unpublished data).



When observing the suppressed control weights of the organisms fed 1 ml YCT/d, as depicted in Figure 6, it is unsubstantiated for USEPA to ascertain that the malnourished organisms in the 2000 USGS test were unlikely to have been stressed to an extent that would have been significant to their reproductive success. The rationale for the acceptability of the 2000 USGS test was provided on page 42 of the criteria document, in which it is stated "*In addition, the average control reproduction (6.4 young/female) also met minimum performance values. Although the feeding rate used in this test was below that recommended for H. azteca exposures lasting longer than 10 days, the finding that control organisms met performance criteria applied in tests using a higher feeding rate supports retaining these data for use in deriving AWQC.*" Appendix K provides recommended performance criteria for *Hyalella azteca* testing, in which it is stated that "*At the end of a 42-day test, control reproduction should average ≥6 young per female. Lower performance may indicate diet/ration may have been limiting.*" While the 2000 USGS study barely surpassed the minimum control reproduction requirements, it is now evident that much greater fecundity can be achieved using Tetramin-based diets. For example, Dr. Soucek found that Tetramin-based diets supplemented with diatoms or wheatgrass resulted in >10 young/female, with a maximum result of 28.4 young/female (Soucek et al. 2016, in press). Please see Table 3 from Soucek et al. 2016 (in press), provided as Attachment 1 within this comment letter, for further documentation of the disparity between the fecundity observed with YCT-based diets and enhanced, present day diets. Additionally, see Figure 1 from Soucek et al. 2016 (in press), inserted below, that depicts the significant relationship (r = 0.90, p <0.0001) between *Hyalella azteca* dry weight/individual and the number of young/female produced.

Figure 1.



The supporting information within this letter clearly suggests that the 2000 USGS test was conducted with suboptimal feeding conditions. Further, it must be noted that all of the supporting information within this letter was gathered from tests that measured the growth rates and fecundity of control organisms that were not exposed to a stressor. Exposing malnourished, stressed organisms to an additional stressor (e.g. cadmium) over the context of a 42 day period would only further impact growth and reproduction compared to the testing of properly fed, healthy organisms exposed to the same stressor.

Test organisms in the 2000 USGS study did not attain minimum growth requirements

Appendix K of the draft 2015 cadmium criteria document includes recommended test conditions and methods for testing with *Hyalella azteca*, in which it is stated that control organism average dry weights should be ≥0.35 mg/individual after 28 days and ≥0.50 mg/individual after 42 days. The average dry weights of controls in the 2000 USGS study were 0.27 mg/individual, which did not surpass minimum growth requirements. Inexplicably, however, USEPA and the study authors concluded that the dry weights measured in the test were inaccurate, therefore the length data obtained from the test were extrapolated to dry weight using a length-weight equation derived for this test organism. Using this equation, the study authors concluded that the average dry weights of the control organisms were 0.52 mg/individual, thus surpassing the minimum requirement of ≥0.50 mg/individual after 42 days. While the criteria document states that "*the same laboratory has developed a robust empirical relationship between amphipod length and weight*", there is no documentation provided for this equation and it is unknown what diet regime the length-weight equation was derived from. Was the length-weight equation specifically developed using data generated from organisms being fed rations of 1 ml YCT/day, or was this equation developed using data generated from organisms being fed rations consistent with present day feeding recommendations? It is conceivable that length-weight equations derived from organisms fed 1 ml YCT/day vs. present day diet recommendations would be significantly different from one another. In regards to the notion that dry weight measurements from the 2000 USGS test were inaccurate, the IEPA is confounded as to how one can underestimate the dry weight of an organism. It is understandable

how an overestimation could be made due to the inadequate drying of test organisms, but an underestimation seems more unlikely to occur, especially considering the expertise of the study authors. It is more plausible that the dry weights from the test were in fact accurate, and that the study organisms were simply underfed and did not achieve the minimum weights required to be a valid test.

Dilution series used in the 2000 USGS test did not appropriately bracket the effect concentration

The dilution series used in the 2000 USGS test consisted of a control treatment and cadmium concentrations of 0.1, 0.3, 0.5, 2.0 and 3.0 µg/L. A standard dilution series results in a doubling of each test concentration, but the 2000 USGS test had a unique dilution series which resulted in a large gap between intermediate treatment concentrations (0.5 and 2.0 µg/L). Unfortunately, the level of effect occurred between these two test concentrations, as the no observable effect concentration and lowest observable effect concentration were 0.5 and 2.0 µg/L, respectively. While the IEPA recognizes that a point estimation technique was used for the 2000 USGS study and that a maximum acceptable toxicant concentration was not derived from the 0.5 and 2.0 µg/L treatments, the IEPA questions what the estimated level of effect would have been had a 1.0 µg/L treatment been added into the dilution series. The IEPA contends that a standard dilution series would have better identified the threshold of effect, which may or may have not resulted in a markedly different Genus Mean Chronic Value (GMCV) of the test organism. The precision of the level of effect ascertained from this study is paramount, as the test result was the sole determinant of the GMCV for *Hyalella azteca*, which is the most sensitive GMCV in the chronic dataset. The IEPA, USEPA, and other states knowledgeable in methods used for national criteria derivation are aware that any small change to the lowest GMCV of a small dataset has a substantial effect on the resulting chronic criterion. Therefore, a small change to the *Hyalella azteca* GMCV due to a refined dilution series could have a substantial decrease or increase on the final chronic criterion for cadmium, thus it is crucial that the precision of the *Hyalella azteca* GMCV be verified with a retest and a more conventional dilution series.

The Illinois EPA contact for these comments is Brian Koch, brian.koch@illinois.gov

Attachment 1

Table 3. Mean (standard deviation) percent survival, reproduction and weight of *Hyalella azteca,* and dissolved oxygen concentration in all treatments from tests #1 through #7. Treatments (trt) ranked from greatest number of young per female to the least. T = Tetramin, D = diatoms, Y = YCT, W = wheatgrass, A = alfalfa, M = maple leaves; Subst. = substrate, U = unconditioned screens, C = conditioned screens; Reprod. = reproduction; DO = dissolved oxygen. X indicates diets were used at rates detailed in table 2. Waters used for each test are shown in table 2.

Test/trt.	Diet						Subst.	Survival	Reprod.	Weight	DO[a]
	T	D	Y	W	A	M		%	# young/female.	mg	mg/L
7/a	1X	X					U	87 (5)	28.4 (2.7)	1.101 (0.039)	6.39 (0.30)
3/e	1X			X			C	94 (12)	18.4 (5.3)	1.047 (0.090)	7.59 (0.66)
6/c	1X	X					U	92 (4)	15.3 (2.9)	0.950 (0.032)	7.07 (0.52)
5/b	1X			X			C	96 (5)	14.6 (3.4)	1.011 (0.050)	7.79 (0.89)
3/c	1X						C	94 (8)	14.3 (1.7)	0.937 (0.092)	7.81 (0.71)
3/g	1X				X		C	94 (8)	14.1 (3.3)	0.972 (0.096)	7.34 (0.53)
6/b	1X			X			C	94 (8)	13.2 (2.7)	1.059 (0.065)	7.38 (0.77)
4/d	1X			X			C	96 (5)	11.0 (1.9)	0.775 (0.142)	7.38 (0.53)
4/c	1X			X			U	90 (6)	6.3 (4.2)	0.620 (0.072)	7.58 (0.65)
4/b	1X						C	76 (8)	6.3 (5.3)	0.723 (0.101)	5.80 (0.48)
1/f	1X*		0.25X				U#	62 (12)	5.6 (3.1)	0.662 (0.109)	7.09 (0.53)
3/b	1X						U	94 (8)	5.1 (2.6)	0.624 (0.038)	6.79 (0.54)
3/a	1X*						U	94 (8)	5.1 (2.9)	0.497 (0.062)	7.72 (0.34)
1/b	1X*						U#	80 (10)	4.1 (4.7)	0.533 (0.133)	7.40 (0.28)
1/c	0.5X*		0.5X				U#	80 (12)	4.1 (2.6)	0.425 (0.099)	7.52 (0.28)
4/a	1X						U	86 (10)	3.4 (1.0)	0.676 (0.060)	6.21 (0.54)
2/f			2.5X		X		U#	82 (12)	3.1 (1.5)	0.338 (0.058)	6.58 (1.47)
1/e			2.5X				U#	74 (23)	2.4 (2.2)	0.320 (0.153)	6.76 (1.00)
5/a	1X			X			U	86 (14)	2.3 (1.1)	0.558 (0.129)	6.81 (1.03)
2/e	1X*				X		U#	86 (14)	2.0 (2.5)	0.490 (0.094)	6.70 (0.91)
2/d			2.5X	X			U#	78 (12)	1.8 (0.8)	0.313 (0.034)	6.56 (1.53)
1/d			2.0X				U#	74 (17)	1.6 (1.9)	0.324 (0.187)	6.91 (1.12)
2/c	1X*			X			U#	94 (8)	1.5 (1.6)	0.439 (0.038)	6.57 (0.76)
1/a			1X				U#	84 (13)	1.4 (0.9)	0.317 (0.066)	7.54 (0.24)
3/f	1X				X		U	76 (5)	1.3 (1.7)	0.358 (0.099)	6.70 (0.79)
3/d	1X			X			U	76 (19)	0.8 (1.0)	0.355 (0.094)	6.42 (0.70)
2/a			2.5X				U#	66 (10)	0.5 (1.0)	0.203 (0.050)	6.73 (1.41)
6/a	1X			X			U	82 (12)	0.5 (0.6)	0.223 (0.037)	6.32 (0.49)
2/b	1X*						U#	86 (13)	0.2 (0.3)	0.388 (0.060)	7.26 (0.60)
2/h			2.5X			X	U#	18 (8)	0.2 (0.2)	0.119 (0.059)	5.98 (0.95)
2/g	1X*					X	U#	10 (6)	0.0 (0.0)	0.084 (0.037)	6.76 (0.44)

[#] unconditioned screens were used, but the same screens were used throughout the test. Treatments marked as U (without #) started with unconditioned screens and screens were replaced throughout the test to eliminate biofilm accumulation.

[*] For Tetramin treatments, an * indicates feeding occurred on Monday, Wednesday, and Friday only. Otherwise, Tetramin was fed daily.

[a] DO values are means of all measurements for a given treatment during the course of the experiment